



IN THE UNITED STATES PATENT OFFICE

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Inventor : W. Roy KNOWLES, M.D.
Filing Date: 19 July 2000
Ser. No.: 09/619,142
Examiner: Vickie KIM
Art Unit: 1614

Honorable Commissioner of
Patents & Trademarks
Washington, DC 20231

APPEAL BRIEF

This Appeal Brief is submitted pursuant to the 5 July 2001 Notification of Non-Compliance.

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I. REAL PARTY IN INTEREST

The real party in interest is the applicant inventor.

II. RELATED APPEALS AND INTERFERENCES

There are no related appeals nor interferences which will directly affect nor be directly affected by nor have a bearing on the Board's decision in this appeal.

III. STATUS OF CLAIMS

Claims 1-22 are pending, stand twice rejected, and are appealed.

IV. STATUS OF AMENDMENTS

There are no claim amendments.



V. SUMMARY OF INVENTION

A. Overview

The invention relates to maintaining healthy hair and preventing abnormal hair loss, by using *minoxidil* or a *testosterone blocker* (e.g., progesterone) together with a skin penetration enhancer.

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B. The Art and Its Shortcomings

Minoxidil in the systemic blood circulation is a potent anti-hypertensive cardiovascular drug. Minoxidil is also used topically as an anti-alopecia agent (e.g., ROGAINE®). It has been understood that topical use with a skin penetrating agent would load the drug into the systemic blood circulation. This risks precipitating cardiac side effects. Such risk is unacceptable for cosmetic use for hair loss. Thus, minoxidil for hair loss has never included penetration enhancer. Specification at 2-7; Knowles, *Rule 132 Declaration*.

Progesterone is a birth control drug. Side effects include carcinogenicity, feminization, and impotency. Progesterone has been disclosed topically for hair loss. Such teaching, however, due to concerns over potential side effects, include a "blocking agent" and never include skin penetration enhancer. Id. at 7.

The foregoing is taught by the references and undisputed by the Examiner.

C. Dr. Knowles' Counter-Intuitive solution

Dr. Knowles has turned this conventional wisdom on its head. He has found that, contrary to the teachings of the art, penetration enhancer can safely be used with minoxidil or a testosterone blocker - if used properly. Specification at 8-9. He tested his invention in rigorous, confidential clinical trials. His invention has been proven **ten times more effective** over prior



art preparations, with **qualitatively better results**, with none of the **adverse side effects** feared in the prior art. *Id.* at 8, 12-14; Knowles, *Rule 131 Declaration*; Knowles, *Rule 132 Declaration*.

The claims are thus drawn to minoxidil or testosterone blocker combined with a specific amount of skin-penetration enhancer. Claim 1 reads:

1. A composition of matter intended for topical use in preventing or treating alopecia, or maintaining healthy hair, said composition of matter comprising:
- a) an active compound selected from the group consisting of: a pharmaceutically or cosmetically effective topical amount of a testosterone blocker and minoxidil, and
 - b) a penetration enhancer, said penetration enhancer present in a concentration sufficient to aid said active compound in penetrating the skin surface to a depth of approximately the depth of hair bulbs.

D. Conclusion

The references of record lack essential claim limitations. The references fail to enable (in fact actively teach away from) the claimed invention. The Applicant has "sworn behind" a key reference. Furthermore, the rejections must be reversed because the examiner refuses to produce an affidavit of references.

Thus, all pending rejections must be reversed.

VI. ISSUES

A. The Issues Presented

Whether Applicant has sworn behind Hoke?

Whether Hoke, assuming it has not been sworn behind, may be combined with Orentreich?

Whether any reference of record includes the claim limitation, "penetrating the skin surface to a depth of approximately the depth of hair bulbs"?

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Assuming the claim limitation, "penetrating the skin surface to a depth of approximately the depth of hair bulbs" is present in a reference, whether the reference enables one of skill in the art to practice that limitation?

B. References Relied on by the Examiner

The Examiner relies on the following references:

1. Hoke + Orentreich

Orentreich, United States Letters Patent No. 5,053,403, teaches the topical use for hair loss of progesterone combined with a "blocking agent." Orentreich says progesterone has systemic side effects so serious that it should not be used for hair loss. Id. at col.1 lines 45-51 ("The serious side effects (such as decreased libido) produced by the systemic administration of antiandrogens precludes the systemic use of these drugs for the treatment of the above skin disorders. For example, progesterone is a highly active 5 α reductase enzyme inhibitor, but systematically disturbs the menstrual cycle in women").

Hoke, Patent No. 5,994,319, similarly teaches progesterone's adverse systemic effects, col.4 lines 18-23. Hoke teaches that minoxidil has "potent" cardiovascular side effects and doesn't work well for hair loss. Id. at col.3 lines 4-14. Hoke advocates instead the use of his claimed antisense oligonucleotides (DNA) for hair loss. Id.

2. Bazzano + Mikulak

Bazzano, Patent No. 5,183,817, teaches using retinoids for hair loss. Bazzano says that minoxidil alone *does not work*. Bazzano says,

Minoxidil is recognized as being somewhat effective in producing new vellus hair growth and sparse terminal hair growth in a pre-selected group of subjects, However, its effect is far from satisfactory in most subjects. * * * [M]inoxidil may not be able to sustain the growth of terminal hairs from vellus hairs on the scalp. In the majority of subjects with alopecia, terminal hair growth on the scalp

may not be initiated or sustained by the topical application of minoxidil nor by its systemic administration.

Id. at col.3, line 53-56 and col.4, lines 49-54. Bazzano teaches that systemic minoxidil presents serious cardiac side effect risks. Id. at col.3 line 49-52; col.43-45.

Mikulak, an abstract of 50(2) J. Pharm. Pharmacol. 153 (1998), discloses a new skin penetration agent (TPDS). The abstract compares it to other methods of drug administration. Mikulak compares (1) TPDS; (2) a 50:50 propylene glycol ("PEG") alcohol vehicle (the control); (3) a "commonly used skin penetration enhancer"; and (4) oral administration ("We compared the TPDS with a 50:50 (vol./vol.) mixt. of propylene glycol and ethanol, a commonly used penetration enhancer, and with oral administration.").

3. Rajadhyaksha

Rajadhyaksha, Patent No. 5,482,965, teaches improved skin penetration agents.

Rajadhyaksha teaches that his compounds effectively deliver drugs into the systemic blood circulation. Id. at col.2 line 16-19. The compounds pass "through the skin and the systemic circulation" to the liver, and yield nontoxic metabolites. Id. "Systemically active agents are used in amounts calculated to achieve and maintain *therapeutic blood levels* in a human or other animal." Id. at col.3 line 53-60; col.10 line 11-14. Rajadhyaksha teaches using his compounds for systemic drug delivery. Id. at col.18 line 1-28. His compounds do in fact make drugs penetrate *completely though the skin*. Id. at col.18 line 55 to col.19 line 12.



VII. GROUPING OF CLAIMS

The following groups of claims are, as explained in Section VIII below,
separately patentable:

- 5 Group I: 1-22
 Group II: 2, 4-11, 13, 15-22
 Group III: 4-11, 15-22
 Group IV: 6, 17
 Group V: 11, 22

VIII. ARGUMENT

10 The Examiner raises three bases for rejection: Hoke, Bazzano, and Rajadhyaksha.

We address each in turn.

A. Hoke + Orentreich

Claims 1-22 stand rejected as obvious over Hoke in view of Orentreich. Hoke cannot render the claims obvious, because (1) the Applicant has sworn behind Hoke, (2) the
15 references provide no suggestion to combine (and in fact teach to not combine) these references, (3) the references lack claim limitations, and (4) the claimed invention shows secondary indicia of non-obviousness.

1. Applicant Swore Behind Hoke

A §103 rejection is overcome by swearing behind any one reference. MPEP
20 §715.02 ¶4. Here, Applicant swore behind Hoke. *See Knowles, Rule 1.131 Declaration.* The Examiner has accepted this Declaration without objection. *See Office Action* at 4-5 (14 March 2001). Thus, the rejection **must** be withdrawn. MPEP §715.02 ¶4.

2. Hoke Teaches Away From The Claims

Hoke teaches using nucleotides. Hoke says nucleotides are safe because they are
25 "highly selective" genetic binders. They thus do not pose the systemic side effect risk seen with

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minoxidil or progesterone. Orentreich teaches using progesterone together with "blocking agents."

Hoke and Orentreich teach away from combining penetration enhancer with minoxidil or testosterone blocker. Hoke says minoxidil is "a potent anti-hypertensive" cardiac drug. Hoke says that systemic use of minoxidil can risk cardiac arrhythmias. Hoke at col.5, line 4-6. Hoke thus teaches that minoxidil combined with a penetration enhancer may precipitate cardiac arrhythmias. Knowles, *Rule 132 Declaration* at ¶¶3-7, 14-17. Hoke also says minoxidil doesn't work. Hoke col.3, line 411 ("only 8% of patients reported a dense re-growth of scalp hair"). Thus, Hoke discourages using minoxidil *at all*, with or without penetration enhancer.

Knowles, *Rule 132 Declaration*.

Hoke teaches progesterone's adverse side effects like "feminization or impotency." *Hoke* at col.4, line 18-24. Orentreich similarly teaches serious systemic side effects such as decreased libido and "systemic[] disrupt[ing] the menstrual cycle in women."

Orentreich concludes that cardiac arrhythmias, feminization and impotency are side effects generally **not acceptable** for treating hair loss. Orentreich says these side effects **preclude systemic use** of these compounds for skin disorders. Orentreich at col.1, line 45-52.

Hoke and Orentreich thus teach that for hair loss, combining a penetration enhancer with minoxidil or progesterone is not worth the risk. They thus teach away from the claimed combination. Knowles, *Rule 132 Declaration*. The *Examiner does not dispute this*.

3. The Examiner's Rationale Is Legally Impermissible

The Examiner accepts the forgoing. What basis, then, does the Examiner offer to combine the references? The Examiner takes "official notice" that progesterone and anti-sense

nucleotides have "the same biological pathway" and "work via same mechanism." Office Action at 3 (5 Oct. 2000). This assertion is baldly incorrect.¹

Anti-sense nucleotides bind to sense nucleotides (genes) coding for 5 α reductase, slowing production of it. Knowles, *Rule 132 Declaration*. In contrast, progesterone
5 competitively binds to the 5 α reductase receptor. The two types of compounds thus use completely different biological pathways; one affects gene translation **inside** the cell, the other affects receptors **outside** the cell. Id. One affects 5 α reductase, the other affects a cell receptor. This is why Hoke himself says these two classes of compounds are *not* "interchangeable" and why Hoke's own compounds are patentably distinct from progesterone. Id.

10 Applicant asked for an affidavit of references to support the Examiner's incorrect assertion. The examiner refuses to respond. Because the Examiner refuses to respond, the rejection **must** be withdrawn. Ex parte Nouel, 158 USPQ 237, 239 (B.P.A.I. 1967) ("when the examiner judicially notices or to show wherein such matter, and such is challenged, *there is reversible error when the examiner fails to cite the well known thing on which he relies*").²

¹ And improper. The suggestion to combine cannot be based solely on an officially noticed fact. See Ex parte Grochowski, No. 95-1343 at 5 (B.P.A.I. June 27, 1995). In re Eynde, 178 USPQ 470, 474 (C.C.P.A. 1973) elaborated, "The facts concerning the state of the art are normally subject to the possibility of rational disagreement among reasonable men and **are not amenable to the taking of [judicial] notice**. If evidence of the knowledge possessed by those skilled in the art is to be properly considered, it must be timely injected into the proceedings."

² In re Ahlert, 165 USPQ 418, 420 (C.C.P.A. 1970), explained some limits of official notice, commenting, "Assertions of technical facts in areas of esoteric technology **must always be supported** by citation to some reference work recognized as standard in the pertinent art. ... Allegations concerning specific 'knowledge' of the prior art ... should also be supported. ... The facts so noticed serve to 'fill the gaps' ... and **should not** comprise the principle evidence upon which rejection is based."

4. Hoke Lacks Claim Elements

The claims require "penetration enhancer present in a concentration sufficient to aid said active compound in penetrating the skin surface to a depth of approximately the depth of hair bulbs." Here, the references lack this limitation. The Examiner **admits** this.

5 The Examiner, however, quips, "penetration enhancer ... is not considered critical." Office Action at 5 (Oct. 24, 2000). The Examiner is not at liberty to simply ignore a claim limitation shown by the Specification and the Inventor's *Rule 132 Declaration* to be essential. Anticipation requires **all** limitations exist in the prior art. *E.g.*, Akzo N.V. v. U.S. Intern. Trade Comm'n., 808 F.2d 1471 (Fed.Cir. 1986), *cert. denied*, 482 U.S. 909.

10 The Examiner alternatively takes official notice combining penetrant and minoxidil "is common practice which has been utilized by the skilled artisan in the state of the art." Office Action at 5 (Oct. 24, 2000). Taking official notice that the claimed invention is known in the art is improper. In re Pardo & Landau, 214 USPQ 673, 677 (C.C.P.A. 1982) (official notice of level of skill in the art is reversible error); In re Spormann, 150 USPQ 449, 452
15 (C.C.P.A. 1966) (Board's official notice of "inherent" teachings of art is reversible error); 37 CFR §1.104(d)(2) (official notice "**must be supported**, when called for by the applicant, by an affidavit from the examiner"). Applicant thus asked for an affidavit of references showing such combinations in common practice and utilized by the skilled artisan. Predictably, examiner refuses to respond. Thus, the rejection **must** be withdrawn. Nouel, *supra*.

20 5. Secondary Indicia of Non-Obviousness

The claimed invention shows many indicia of non-obviousness. The claimed combination solves a long felt need for a solution to a hairy, difficult problem. Dr. Knowles succeeded where others failed. Dr. Knowles' results were unexpectedly superior, showing *ten times* the effectiveness of topical minoxidil, with *qualitatively better* results. Knowles, *Rule 132*

Declaration. The Examiner accepted this secondary evidence without dispute. Thus, the invention should not be found obvious.

B. Bazzano + Mikulak

Claims 1-4, 8-10, 12-5 and 19-21 stand rejected over Bazzano. Bazzano,
5 however, fails to teach *certain claim limitations*, and does not *enable* the claims.

1. Bazzano Lacks Limitations for Claims 1-22

Bazzano does not teach skin penetrant. *The Examiner admitted this*, but quipped that this claim limitation "is not considered to be critical." Office Action at 5 (Oct. 24, 2000). The Examiner cannot simply ignore a claim limitation. Because Bazzano does not disclose it,
10 Bazzano cannot anticipate claims 1-22. *E.g., Akzo, supra.*

Claims 2, 4-11, 13, and 15-22 further require a "testosterone blocker." It is undisputed that Bazzano completely lacks this claim limitation.

2. PEG-Ethanol is not a "penetration enhancer"

Claim terms are interpreted in light of the specification. Here, the Specification
15 defines "penetration enhancers" (pg. 9-11) and "carrier vehicles" (pg. 17-20). PEG and ethanol, "alone or in combination," *id.* at 17, 18-19, are defined as "carrier vehicle," not a "penetration enhancer." In other words, the claim term "penetration enhancer" excludes PEG-ethanol. Thus, as a matter of law, PEG-ethanol cannot anticipate the claim limitation "penetration enhancer."

Bazzano teaches minoxidil in a propylene glycol ("PEG")-ethanol vehicle.³ This
20 teaching is not controversial; long before Bazzano, PEG-ethanol has been used as an inert cosmetic vehicle. It has been used for years, for ROGAINE® brand topical minoxidil and a

³ See Bazzano at Example I. The Examiner also says Example II teaches PEG-ethanol. Office Action at 3 (March 14, 2001). It does not.

variety of other cosmetics.⁴ PEG-ethanol is available commercially as an inert carrier vehicle (e.g., The Neutrogena Company's VEHICLE/NTM, discussed in the Specification).

The Examiner, however, ignores the clear definition in the Specification and says PEG-ethanol is "a commonly used penetration enhancer." Office Action at 3-4.

5 It is not. The Examiner's own references say so. Bazzano, at claim 24, calls PEG-ethanol an inert "vehicle." Knowles, *Supplemental Declaration*. Likewise, Mikulak teaches PEG-ethanol is an inert vehicle used as the experimental control (Mikulak discloses a comparison of (1) TPDS; (2) 50:50 PEG-ethanol; (3) a "commonly used [albeit unidentified] skin penetration enhancer"; and (4) oral administration). *Id.*

10 Further, Bazzano teaches the PEG-ethanol mix used in ROGAINE®. *Id.* This PEG-ethanol vehicle was tested against the claimed compounds and shown to be only one tenth as effective, and cannot produce the qualitatively superior results of the claimed compounds. *See* Specification; Knowles, *Rule 132 Declaration*. This is undisputed.

15 3. Bazzano Does not Enable The Claims

To anticipate, Bazzano must enable one of skill in the art to practice the claimed invention. Biogen Inc. v. Amgen Inc., __ F.3d __, __ (D.Mass. 1999). Here, Claim 1 requires "penetration enhancer present in a concentration sufficient to aid said active compound in penetrating the skin surface to a depth of approximately the depth of hair bulbs." It is undisputed that Bazzano does not enable this.

⁴ The Food & Drug Administration monograph on sunscreens treats PEG 5% as an inert vehicle. *See* 21 C.F.R. 352.70.

Further, it is undisputed that Bazzano actively teaches away from the claimed invention. Bazzano says that without added retinoid, minoxidil just does not work. *Supra* at §I.C.2; Knowles, *Rule 132 Declaration* at ¶11. The examiner accepts this without objection.

Because Bazzano lacks claim limitations, and because it is undisputed that
5 Bazzano actively teaches away from the claimed invention, the rejection must be withdrawn.

C. Rajadhyaksha

Claims 1-4, 8-10, 12 and 14 stand rejected as anticipated by Rajadhyaksha. Rajadhyaksha cannot anticipate the claims, because Rajadhyaksha lacks several claim limitations, and does not enable the claimed invention.

10 1. Rajadhyaksha

Rajadhyaksha teaches skin penetration agents. Rajadhyaksha teaches they deliver drugs into the systemic circulation. The reference teaches delivering drugs **completely through the skin** into the systemic bloodstream. Id. at Example 32. Knowles, *Supplemental Declaration*. The reference says:

15 Typically systemically active agents which may be delivered transdermally are therapeutic agents which are sufficiently potent such that they can be delivered
20 *through the skin or other membranes to the bloodstream* in sufficient quantities to produce the desired therapeutic effect. In general this includes agents in all of the major therapeutic areas including ... cardiovascular preparations including calcium channel blockers, beta-blockers, antiarrhythmics, antihypertensives, diuretics, vasodilators including general, coronary, peripheral and cerebral.

Id. at col.7 line 40-57.

The reference cannot anticipate the claimed invention, because the reference lacks
25 various claim limitations and fails to enable the claimed invention.

2. Rajadhyaksha Lacks Essential Claim Limitations

Rajadhyaksha lacks limitations of various claims. The following groups of claims are thus separately patentable:

a) *Claims 1-22*

5 These claims are limited to:

- b) a penetration enhancer, said penetration enhancer present in a concentration sufficient to aid said active compound in penetrating the skin surface to a depth of approximately the depth of hair bulbs.

10 Rajadhyaksha does not teach this claim limitation. Rajadhyaksha does not teach delivering drugs "to the depth of the hair bulbs." To the contrary, Rajadhyaksha teaches delivering drugs **completely through the skin**, into the systemic bloodstream. *Id.* at Example 32. Rajadhyaksha teaches delivering drugs to the systemic blood stream using a 5% concentration of 5-Amino-5-ethyl-2-(3-heptyl)-1,3-dioxane ("A5A"). *Id.* at Examples 28-30. Rajadhyaksha teaches
15 delivering minoxidil to the blood stream using 5% 5A5. *Id.* at col.15 line 26. Perhaps because he feared the dangerous cardiac side effects, Rajadhyaksha avoided using this example -it is just a prophetic example. Because Rajadhyaksha lacks the claim limitation, Rajadhyaksha cannot anticipate claims 1-22.

b) *Claims 2, 4-11, 13, 15-22*

20 These claims require "intended for topical use in preventing or treating alopecia." Rajadhyaksha teaches (at Example 29), a systemic birth control patch for delivering progesterone to the systemic blood stream. Knowles, *Supplemental Declaration*. This patch is not used in "alopecia or maintaining healthy hair," nor does it appear to deliver testosterone blocker "to a depth of approximately the depth of hair bulbs." This is uncontested. Because it is uncontested
25 that Example 29 lacks these limitations, it cannot anticipate these claims as a matter of law.

c) *Claims 4-11, 15-22*

These claims require both minoxidil **and** testosterone blocker. The reference does not disclose this combination. This is uncontested. The Examiner, however, appears to misread the claims to require "either minoxidil **or** progesterone." Office Action at 2, line 20 (27 March 2001).

d) *Claims 6 and 17*

These claims require that "the penetration enhancer is trimethyl acetate." The reference expressly teaches away from using trimethyl acetate, teaching instead the use of Rajadhyaksha's compounds. This is undisputed.

e) *Claims 11 and 22*

These claims additionally require sunscreen. No reference of record suggests the combination of sunscreen with penetration enhancer. This is undisputed.

3. *The Reference Is Not Enabling*

For hair loss, the art of record teaches away from combining penetration enhancers with minoxidil or testosterone blocker, due to adverse systemic side effects. This is undisputed. The Examiner, however, notes that the combination -if made- would benefit by using an improved penetration agent. Office Action at 2, lines 18-22 (14 March 2001). While this assertion may be correct, it is inapposite. ***It is uncontested*** that the reference does not enable the claimed invention. Knowles, *Supplemental Declaration*.




IX. SUMMARY

The art of record completely lacks certain claim limitations. It is undisputed that the art of record does not enable the claimed invention. Further, Applicant has "sworn behind" a key reference. The Examiner has refused to produce an affidavit of references supporting factual assertions. Thus, the pending rejections must be withdrawn, as a matter of law.

Please find enclosed (i) an Appendix of references relied on; (ii) two additional copies of this Appeal Brief; and (iii) the Supplemental Declaration.

Respectfully submitted,


Mark POHL, Reg. No. 35,325
18 July 2001

PHARMACEUTICAL PATENT ATTORNEYS,
POHL & ASSOC., LLP
55 Madison Avenue, 4th floor (P 4014)
Morristown, NJ 07960 USA
Direct: licensinglaw@juno.com
☎ +1 (973) 665-0275

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X. CLAIMS ON APPEAL

1. A composition of matter intended for topical use in preventing or treating alopecia, or maintaining healthy hair, said composition of matter comprising:
 - 5 a) an active compound selected from the group consisting of: a pharmaceutically or cosmetically effective topical amount of a testosterone blocker and minoxidil, and
 - b) a penetration enhancer, said penetration enhancer present in a concentration sufficient to aid said active compound in penetrating the skin surface to a
10 depth of approximately the depth of hair bulbs.
2. The composition of claim 1, wherein said active compound comprises a testosterone blocker.
3. The composition of claim 1, wherein said active compound comprises minoxidil.
4. The composition of claim 3, further comprising a testosterone blocker.
- 15 5. The composition of claim 4, wherein the ratio of penetration enhancer to testosterone blocker to minoxidil in the composition is approximately 5 drops : 0.5 grams : 1 gram.
6. The composition of claim 4, wherein said penetration enhancer is trimethyl acetate and wherein said testosterone blocker is progesterone.
7. The composition of claim 5, wherein said testosterone blocker is present in a
20 concentration of 0.5 grams per 4 ounces of finished liquid.
8. The composition of claim 4, labeled for topical cosmetic use in maintaining normal, healthy hair.

9. The composition of claim 4, labeled for topical pharmaceutical use in preventing or treating a disease.
10. The composition of claim 9, wherein said disease comprises alopecia.
11. The composition of claim 4, further comprising a sunscreen in an amount effective to
5 screen radiation.
12. A method for preventing or treating alopecia, or maintaining healthy hair, said method comprising:
- a) Topically administering an active compound selected from the group consisting of: a pharmaceutically or cosmetically effective topical amount of a
10 testosterone blocker and minoxidil, together with
 - b) a penetration enhancer, said penetration enhancer present in a concentration sufficient to aid said active compound in penetrating the skin surface to a depth of approximately the depth of hair bulbs.
13. The method of claim 12, wherein said active compound comprises a testosterone
15 blocker.
14. The method of claim 12, wherein said active compound comprises minoxidil.
15. The method of claim 14, wherein said active compound further comprises a testosterone blocker.
16. The method of claim 15, wherein the ratio of penetration enhancer to testosterone
20 blocker to minoxidil in the composition is approximately 5 drops : 0.5 grams : 1 gram.
17. The method of claim 15, wherein said penetration enhancer is trimethyl acetate and wherein said testosterone blocker is progesterone.

18. The method of claim 16, wherein said testosterone blocker is present in a concentration of 0.5 grams per 4 ounces of finished liquid.
19. The method of claim 15, labeled for topical cosmetic use in maintaining normal, healthy hair.
- 5 20. The method of claim 15, labeled for topical pharmaceutical use in preventing or treating a disease.
21. The method of claim 20, wherein said disease comprises alopecia.
22. The method of claim 15, further comprising a sunscreen in an amount effective to screen radiation.



XI.

REFERENCES CITED

Roy KNOWLES, M.D., "Hair Loss Prevention"
S.N. 09/619,142, Group 1614

[54] **COMBINATION THERAPY FOR ANDROGENIC ALOPECIA WITH ANTISENSE OLIGONUCLEOTIDES AND MINOXIDIL**

[75] Inventor: Glenn D. Hoke, Jr., Mount Airy, Md.

[73] Assignee: Dyad Pharmaceutical Corporation

[21] Appl. No.: 08/837,190

[22] Filed: Apr. 14, 1997

Related U.S. Application Data

[60] Provisional application No. 60/015,488, Apr. 15, 1996.

[51] Int. Cl.⁶ A61K 48/00

[52] U.S. Cl. 514/44; 435/6; 514/2; 436/501

[58] Field of Search 435/6; 436/501; 514/44; 536/22.1, 23.5, 24.5, 24.3-24.33; 935/77, 78

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,422,262 6/1995 Andersson et al. 435/240.1

OTHER PUBLICATIONS

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New England Biolabs Catalog (1986/87) [Published by New England Biolabs, Beverly, MA, USA], pp. 60-62.

"Finasteride Merck & Co submitted for approval", R&D Focus Drug News, Apr. 7, 1997.

"Merck & Co's Propecia Shows Promise in Hair Loss", Marketletter, Mar. 31, 1997.

Primary Examiner—Ardin H. Marschel

Attorney, Agent, or Firm—Max Stul Oppenheimer

[57] **ABSTRACT**

Minoxidil has been shown to stimulate hair growth or inhibit the loss of hair in a number of patients beginning to develop androgenic alopecia. The mechanism by which minoxidil (2,4-pyrimidinediamine, 6-(1-piperidiny)-3-oxide) alters the hair growth cycle is uncertain, but is thought to act by increasing vascular circulation to the hair follicle. Inhibitors of steroid metabolism, particularly those that inhibit the conversion of testosterone to dihydrotestosterone, have shown effects on hair cycles, including inhibition of hair loss. One class of enzymes targeted by these inhibitors are the steroid 5- α reductases. Minoxidil used in conjunction with effectors of steroid metabolism, leads to enhanced hair growth and decreased rates of hair loss. This specification relates to the use of antisense oligonucleotides targeting 5- α reductases used in conjunction with other hair growth enhancers and/or hair loss inhibitors.

6 Claims, 4 Drawing Sheets

Elevated DHT levels cause conversion of anagen hair to telogen hair in androgenic alopecia

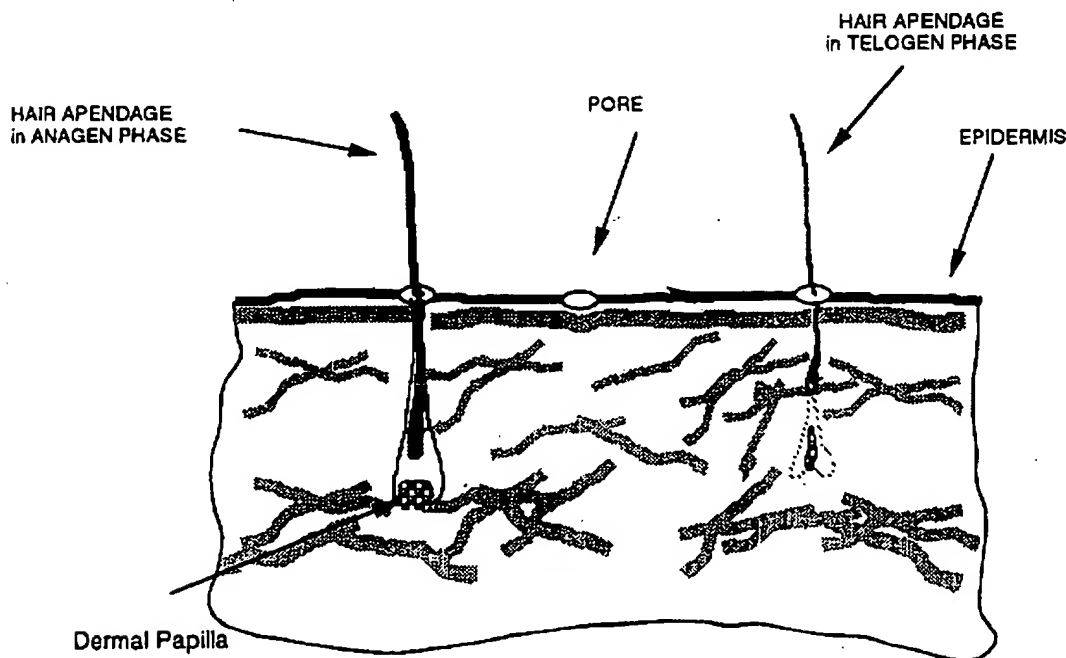


Figure 2. Inhibition of hair loss by antisense targeting 5-alpha reductases and minoxidil

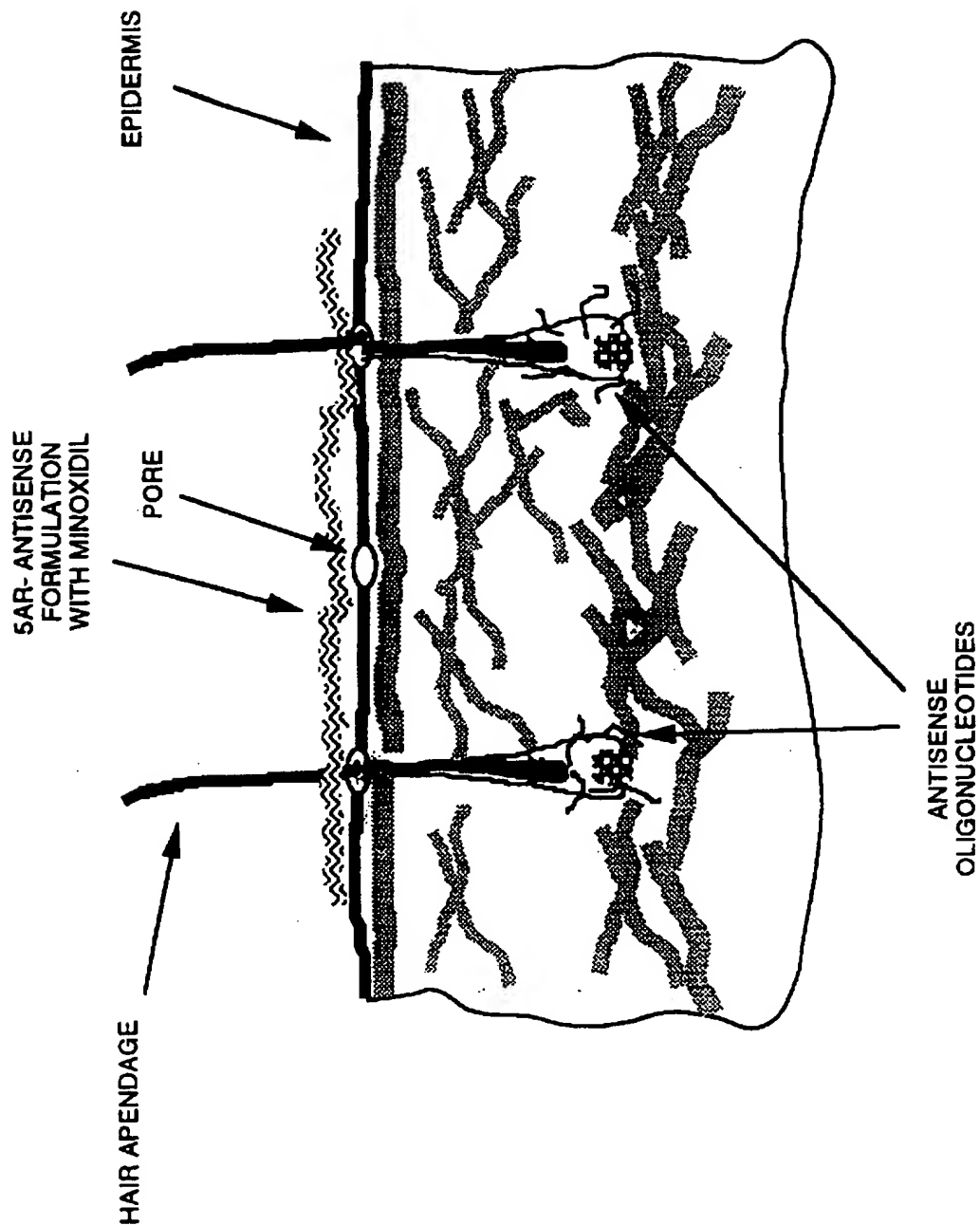
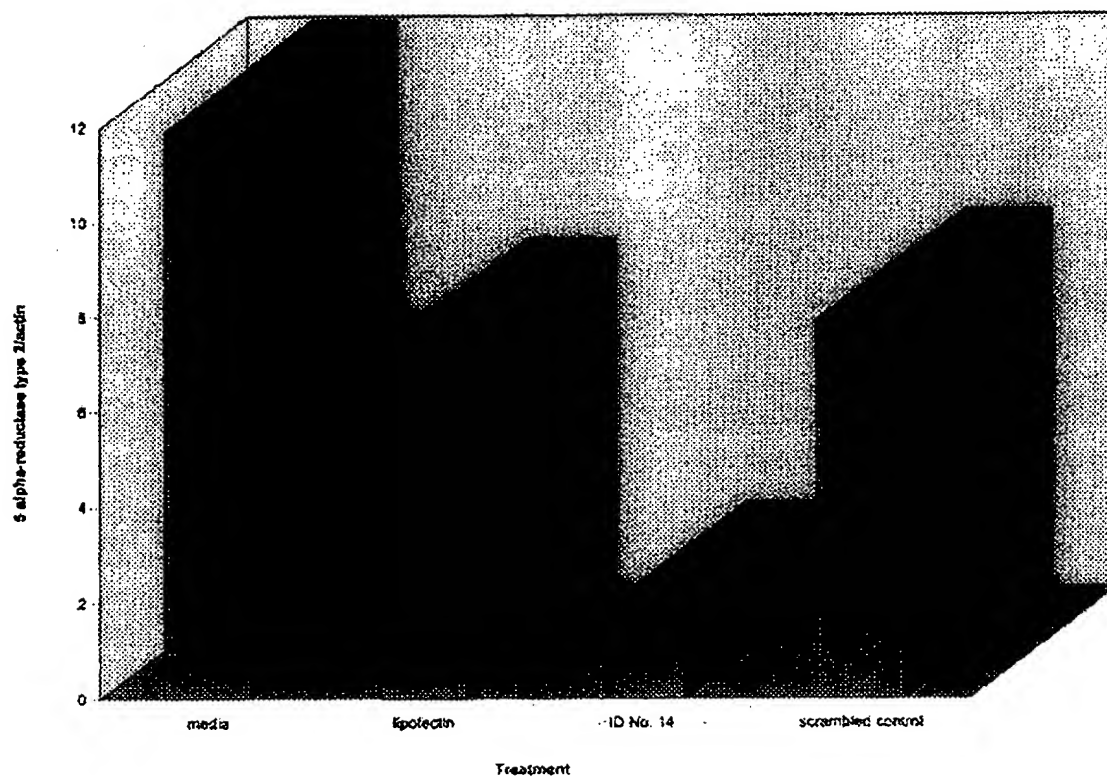


Fig 4 Inhibition of 5 alpha-reductase type 2 by
antisense oligonucleotide ID No.14



ing in the reversal of the balding process. (Marketletter Mar. 31, 1997, "Merck & Co's Propecia Shows Promise in Hair Loss").

The Food and Drug Administration has approved the use of a 2% solution of minoxidil as a topical treatment for male pattern baldness. Through a twice daily application, only 8% of patients reported a dense regrowth of scalp hair, while closer to 33% experienced a moderate regrowth. An additional 33% experiencing little or no regrowth or the growth of vellus hair only and the remaining patients experienced no change in their rate of hair regrowth. The mechanism by which minoxidil stimulates hair growth is unknown. Minoxidil is not thought to affect hair growth or loss by acting at the level that androgens exert their effects.

The use of a combination treatment for androgenic alopecia has been suggested for increasing the effectiveness of minoxidil used alone. For example, the combination of minoxidil treatment with finasteride, a 5-alpha reductase inhibitor, demonstrated that, in combination, these two drugs increased the rate of hair regrowth when compared to either drug administered alone (Diani, A. R. et al., 1992, *J. Clin. Endocrinol. Metabol.* 74: 345-350).

Antisense oligodeoxynucleotides or ribozymes have been successfully employed to decrease mRNA translation (van der Krol, et. al., 1988; Cohen, 1991; Calabretta, 1991; Calabretta, et. al., 1991; Saison-Behmoraras, et. al., 1991). Once the oligonucleotides are taken up by the cells they can elicit an antisense effect by binding to the correct sequences on the target mRNA. The concept behind antisense therapy is based on the assumption that antisense oligonucleotides are taken up by cells and interact with a specific mRNA resulting in the formation of a stable heteroduplex. The interaction of the antisense oligonucleotide with its target mRNA is highly specific and is determined by the sequence of bases complementary to the antisense oligonucleotide as determined by Watson/Crick base pairing.

The development and progression of androgenic alopecia is associated with the local accumulation of DHT. The enzyme steroid 5 α -reductase type 1 is expressed in the inner epithelial sheath of the hair follicle (Wolfgang, E. et al., 1994). This enzyme functions to catalyze the conversion of testosterone to dihydrotestosterone. It would appear that the inhibition of steroid 5 α -reductase type 1 expression, alone or in combination with other agents that decrease steroid 5 α -reductase activity (i.e. Propecia®) or through the inhibition of the expression of other steroid 5 α -reductase genes, would be an effective means for treating androgenic alopecia. It is possible to lower the intracellular concentration of 5 α -reductase by reducing the expression of 5 α -reductase. Thus, it would be possible to inhibit the conversion rate of testosterone to DHT via 5 α -reductase.

Antisense oligonucleotides used for therapeutic purposes were first proposed in 1978 by M. L. Stephenson and P. C. Zamecnik (PNAS 75: 280-284). The concept behind antisense therapy relies on the ability of antisense oligonucleotides to be taken up by cells and form a stable heteroduplex with the target mRNA, thereby down regulating the targeted protein's synthesis.

It has been demonstrated in a number of systems by a number of investigators that oligonucleotides containing an antisense sequence targeting a portion of a particular mRNA are capable of hybridizing to the mRNA and inhibiting the translation of the transcript.

The interaction of an antisense oligonucleotide with target mRNA is highly specific, as hybridization is determined by the sequence of bases complementary to the antisense oli-

gonucleotide (Watson/Crick base pairing of the two strands of nucleic acid). This results in multiple points of contact between the antisense oligonucleotide and the mRNA target, which increases the specificity for hybridization to the correct sequence.

Evidence for down regulation of protein synthesis by antisense oligonucleotides has been well documented in vitro (for reviews see van der Krol, A. R., et al. *BioTechniques* 6: 958-976, 1988; Milligen et. al. *J. Med. Chem* 36:1923-1937, 1993). In vivo studies using antisense oligonucleotides have demonstrated that injection of radiolabeled antisense oligonucleotides into the blood of mice results in distribution of full-length labeled oligonucleotide to the various tissues. Once in the tissue, oligonucleotides can elicit an antisense effect by binding to the correct mRNA and, thus, be suitable for a therapeutic (Miller, P. S. and Ts'o, P. O. P. *Anticancer Drug Design* 2: 117-128, 1987).

More specifically, antisense oligonucleotides targeting 5-alpha reductases have demonstrated the capacity to effectively reduce the synthesis of 5-alpha reductase types 1 or 2. These inhibitors are extremely potent, highly selective, and should not exhibit any of the side effects produced by the anti-androgens (i.e., feminization or impotency).

SUMMARY OF THE INVENTION

It is an object of the invention to provide a treatment to reduce hair loss.

It is a further object of the invention to provide a process for restoring hair.

These and other objects are achieved by providing a process for treatment of androgenic alopecia through inhibition of the expression of human steroid 5 α -reductase type 1. Specifically, this invention relates to the use of oligonucleotides, or other chemical compositions that interact in a sequence specific manner to either the steroid 5 α -reductase type 1 gene, pre-mRNA or mRNA so as to reduce the transcription or translation of the enzyme used in combination with other agents that reduce the rate of hair loss or increase the rate of hair growth. Clinically, such antisense oligonucleotides could be administered alone or in combination with other agents that decrease steroid 5 α -reductase activity (i.e. finasteride) or those having other positive effects in the treatment of androgenic alopecia (i.e., minoxidil). The use of such antisense oligonucleotides to control the expression of steroid 5 α -reductase type 1, offers the potential of developing highly specific and efficacious therapies for the adjunctive treatment of diseases characterized by the local over production of DHT. Thus, through the use of antisense inhibitors of 5-alpha reductase expression, it would be possible to provide an increased benefit to patients being treated with minoxidil for androgenic alopecia.

In a preferred embodiment of the invention, therapeutically effective amounts of oligonucleotides can be administered to a patient so as to substantially block the tissue-specific transcription of the human steroid 5 α -reductase type 1 (or type 2) gene or the translation of the steroid 5 α -reductase type 1 (or type 2) mRNA transcript, thereby substantially reducing the levels of dihydrotestosterone (DHT) in the patient who expresses a condition characterized by the local over production of DHT. It has been demonstrated recently that the use of finasteride (a 5-alpha reductase which blocks the conversion of testosterone to dihydrotestosterone) miniaturizes scalp hair follicles, resulting in the reversal of the balding process. (Marketletter Mar. 31, 1997, "Merck & Co's Propecia Shows Promise in Hair

the 3'-untranslated region of the steroid 5 α -reductase type 1 or 2 mRNA transcripts. In addition to the sequences described above, other sequences contained within the 5 α -reductase transcripts are targeted. This strategy has been adopted because, as yet, there is no method currently available that can predict, with precision, sequences that will become effective therapeutics. Moreover, this invention further contemplates antisense oligonucleotides made complementary to any portion of the steroid 5 α -reductase genes and which are capable of cross-linking DNA, intercalating DNA or binding more tightly by mechanisms such as, for example, triple stranding. Furthermore, the invention contemplates that any oligonucleotide capable of substantially inhibiting the expression of steroid 5 α -reductase type 1 or 2 can be used.

Oligonucleotides of varying lengths have been successfully used to inhibit gene expression. For example, in U.S. Pat. No. 4,806,463 oligonucleotides ranging in size from 12 bases to 26 bases were shown to be incorporated by cells and to be capable of inhibiting the expression of a target mRNA.

In order for the described antisense oligonucleotides to function therapeutically, the oligonucleotides or modified oligonucleotides must be taken up by the cell that expresses the target gene, pre-mRNA, or mRNA. The oligonucleotides of the present invention are constructed so as to insure that the oligonucleotide will pass through the plasma membrane and achieve an intracellular concentration that is sufficient to decrease the expression of steroid 5 α -reductases. Oligonucleotides that are constructed to bind to the steroid 5 α -reductase type 1 or 2 genes are further modified, if necessary, to enable them to pass through the nuclear membrane in levels that are sufficient to reduce transcription. Recent attempts at enhancing the cellular uptake of antisense oligonucleotides have employed a wide variety of techniques including the use of lipoproteins, (de Schmidt, et. al., 1991), and a wide variety of conjugates, such as poly-L-lysine and cholesterol (Goodchild, 1990). Conjugation of cholesterol to the 5' end of an oligonucleotide has been reported to result in a molecule that exhibited reduced serum clearance due to reduction in renal excretion, compared to that observed with control oligo deoxynucleotides (ODNs) (de Schmidt, et. al., 1991). As a result, the conjugation of cholesterol to ODNs may allow an increase in the delivery of drug to liver cells via the LDL transport mechanism. Liposomes containing antisense oligonucleotides can also be targeted to specific cell types by the addition of cell-specific antibodies (Leonetti, et. al., 1990). These and other methods of achieving and maintaining adequate intracellular concentrations of the oligonucleotides are contemplated by this invention and include other methods and compositions that have the capacity to enhance cellular uptake or decrease the efflux of internalized oligonucleotides. Such modifications should not alter the specificity of the oligonucleotide for its target sequence.

The oligonucleotides of this invention comprise predetermined sequences of DNA ranging in size from about 3 bases up to about 100 bases, which is sufficient to define a unique sequence in one of the human steroid 5 α -reductase target transcripts. Less than 10 bases may be used, however the degree of sequence specificity for the mRNA transcripts that encode human steroid 5 α -reductases decreases rapidly with decreasing lengths of the oligonucleotides. On the other hand, oligonucleotide sequences greater than about 100 bases may be subject to decreased uptake by cells. It is preferable that the oligonucleotides comprise about 12 to 26 bases. In a most preferred embodiment a 15 to 25-mer oligonucleotide is used.

Antisense oligonucleotides that are intended for use as drugs must achieve sufficient concentrations in order to decrease the expression of a target protein in a manner that provides therapeutic benefit. The oligonucleotides contemplated in this invention are constructed, or otherwise modified, so as to increase their stability by enhancing resistance to various degradative enzymes (e.g., nucleases). Such modifications will function to permit the concentration of the oligonucleotide therapeutic to be maintained at a level that is sufficient so as to realize therapeutic benefit but cannot substantially alter the specificity of the oligonucleotide for its target sequence. Modifications that improve oligonucleotide stability or efficacy include but are not limited to modifications to the phosphate backbone, termini, sugar moieties and the individual nucleic acid bases. Conjugations to peptides, proteins, carbohydrates, lipids, vitamins or any other conjugation that increases therapeutic potency or efficacy can also be used. Also, any modifications resulting in stable secondary structures including circularization of the oligonucleotide and target sequence, and intrastrand joining of the 3' to the 5' termini through covalent bonds or hybridization and triple stranded binding to mRNA can also be made. Any modifications that reduce nuclease sensitivity while substantially maintaining the affinity and substrate specificity and solubility exhibited by unmodified oligonucleotides are within the scope of the invention.

Several chemically modified oligonucleotides have been developed which substantially block or improve resistance to nuclease activity. These oligonucleotide modifications include phosphorothioate oligonucleotides wherein one of the phosphate oxygens is replaced by sulfur. Another type of modification of oligonucleotides is accomplished by replacing the charged phosphate oxygen with a methyl group or other alkyl group. These nonionic DNA analogs include, for example, methyl phosphonates, alkyl-phosphorothioates, and O-alkyl phosphotriesters. A preferred O-alkylphosphotriester is O-methylphosphotriester. Other DNA backbone modifications at the phosphate group include for example, phosphorodithioate, and phosphotriester oligonucleotides or oligonucleotides based on protein-nucleic acid structures or morpholino-like structures.

Various chemical modifications to either or both the 3'- or 5'-termini and the individual nucleic acid bases are known to improve stability of oligonucleotides to nucleases, stabilize the interaction of oligonucleotides with their specific target molecule, or enhance uptake of the oligonucleotides by cells. Moreover, chemical modifications to the 3' or 5' termini or modifications internal to the oligonucleotide can also be introduced as reporter molecules for example, to allow tracking of the oligonucleotide or as lipophilic moieties to enhance cell uptake. Such molecules can be introduced to both unmodified and backbone modified synthetic oligonucleotides. These moieties can be introduced for example, through thio or amino linkages to terminal hydroxyl or phosphate groups or to specific bases.

Other modifications to the oligonucleotides contemplated in this invention include for example, DNA intercalators, photochemically activated cross-linking or cleaving agents, alkylating agents and redox active nucleic acid cleaving groups.

In vivo and in vitro studies of the degradation of chemically modified oligonucleotides have clearly illustrated that modifications to the phosphate backbone, termini, sugar moiety and individual nucleic acids improve oligonucleotide efficacy or stability or both (Goodchild, 1990). Moreover, acute toxicity studies in mice have demonstrated that some modified oligomers are tolerated at about the same concentrations without undesirable side effects as unmodified oligomers.

of 0.001 to 10.0 mM. In the transient transfection assay, the oligonucleotides are either co-transfected with the steroid 5 α -reductase cDNA or added to the medium at a concentration of 0.001 to 10.0 mM following transfection. Cells are plated in multi-well tissue culture plates. The size of the well used for a particular assay is determined by the level of steroid 5 α -reductase expressed by a given cell line.

The substrate is prepared by dissolving unlabeled testosterone (Sigma Chemical Co., St. Louis, Mo.) in absolute ethanol followed by the addition of either [7-³H] (N)-testosterone (23.3 Ci/mmol) or [¹⁴C]-testosterone (50 mCi/mmol) (New England Nuclear, Boston, Mass.). The solvent is evaporated under a stream of nitrogen and the steroids reconstituted in an appropriate medium.

The medium in the sample wells is aspirated and replaced with fresh medium containing the radiolabeled substrate. An additional three wells containing medium and substrate but no cells is also included in order to account for the non-enzymatic metabolism of the substrate. The plates are returned to the incubator and incubated for an appropriate incubation period that is again dependent on the level of steroid 5 α -reductase expressed by the cell line.

At the end of the incubation period the medium is collected and transferred to an extraction tube containing 5 ml of toluene-ethanol (9:1), to which has been added 40–250 mg each of unlabeled carrier steroids (estriol, estradiol, estrone, 5 α -androstan-3 α ,17 β -diol, 5 α -androstan-3 β ,17 β -diol, 4-androstene-3,17-dione, 5 α -androstan-3,17-dione, testosterone, and 5 α -dihydrotestosterone (Steraloids, Inc. Wilton, N.H.). Depending upon the method used to detect the radiolabeled steroids the extraction solvent may or may not contain 1,000 and 10,000 dpm of [4-¹⁴C]-dihydrotestosterone (steroid 50–60 mCi/mmol) and [4-¹⁴C]-testosterone (50 mCi/mmol) (New England Nuclear, Boston, Mass.); respectively. In assays that employ [7-³H] (N)-testosterone as a substrate, the [¹⁴C]-steroids are included as recovery standards to quantify procedural losses. A small amount of NaCl is also added to the extraction tubes to prevent foaming. The samples are vortexed for approximately 30 seconds and then centrifuged for 10 minutes at 500x g. The organic phase is collected and the solvent evaporated. The steroids are then reconstituted in dichloromethane-methanol (9:1) and analyzed by thin layer chromatography.

The extracted samples are applied to silica gel 60F₂₅₄, 0.25 mm thick, thin layer chromatography plates (EM Science, Cincinnati, Ohio). The plates are developed in a solvent system consisting of chloroform-ethyl acetate (3:1, Mallinckrodt Inc. Paris, Ky.). The plates are allowed to develop until the solvent front migrates to within 2.0 cm of the top of the plate. After removal from the tanks the plates are air dried. The plates are then viewed under 254 nm UV light and the visible spots marked. The plates are then sprayed with primulin (0.001% in acetone-water (4:1) according to the method of Wright (Moore and Wilson, 1975) which allows the identification of additional steroids under 365 nm UV light. The spots are scraped from the plate using a glass wool plugged Pasteur pipette attached to a vacuum line. The steroids are eluted directly into scintillation vials by the addition of 0.2 ml of dichloromethane followed by two washes of 2.0 ml of methanol. The organic solvent is evaporated, and 10.0 ml of scintillation fluid (Ready Organic, Beckman Instruments, Inc. Fullerton Calif.) are added. Samples are analyzed by liquid scintillation spectrometry. In assays that employ [¹⁴C]-testosterone as the substrate, steroid metabolism is analyzed directly using the PhosphorImager imaging system (Molecular Dynamics, Inc., San Jose, Calif.).

Following removal of the media for extraction, the cells are washed with phosphate buffered saline (PBS, pH 7.4), and then harvested by exposure to a trypsin-EDTA solution (0.025% trypsin, 0.265 mM EDTA). The cells are collected and centrifuged at 1400x g for 5 minutes. The supernatant is decanted and the cells resuspended in PBS. An aliquot of the cell suspension is counted in a Coulter Counter Model ZM (Coulter Electronics, Ltd., Luton Beds, England). The remaining cells are sonicated and the protein determined according to the method of Bradford (Bradford, 1976). Corrections are made for procedural losses, and the data expressed as percent inhibition based on steroid concentration in terms of picomoles per mg protein or picomoles/10⁵ cells.

Example 3

It is possible to analyze the proteins of treated and control cells using SDS- denaturing polyacrylamide gel electrophoresis followed by the transfer of resolved proteins to a solid support (Western transfer), such as nitrocellulose, or other filter support. Filter blots are blocked with non-specific proteins and then analyzed using a primary antibody that recognizes either 5- α reductase type 1 or 2. Following the primary antibody, the blots are then reacted with a second antibody that binds to the first antibody and has incorporated with in the second antibody a reporter moiety (i.e., radioactivity, enzyme linked, or other easily detectable reporter group). Following treatment with the second antibody, the blot is analyzed for the amount of 5- α reductase present in a given amount of cellular protein. Thus, it is possible to actually quantitate the amount of 5- α reductase being expressed in cells.

Chinese hamster ovary cells transfected with the human 5 α R-I gene (CHO 1827) or the human 5- α R-II gene (CHO 1829) are plated at 8x10⁶ cells per well, in 6 well dishes in DMEM/F12(1:1) and 5% Fetal Bovine Serum (FBS). Cells are dosed with ODN's (containing 5 ug/ml LipofectinTM, Life Technologies, Inc. Gaithersburg, Md.) in serum-free Opti-MEM for 3 hr at 37° C. and then returned to serum-containing media. Dosing is carried out for three consecutive days due to the long half-life of the 5 α R proteins. Cells are harvested by detachment in PBS with 5 mM EDTA, pelleted, and lysed in a NP40-RIPA buffer. Cellular proteins (5 ug) are then separated by SDS-PAGE on a 12% acrylamide gel before transferring onto a PVDF membrane. The amount of the 5- α R-I or the 5 α R-II proteins and actin are then detected by direct visualization using ECL-Western (Amersham, Arlington Heights, Ill.) and antibodies specific for the proteins. Actin is included as an internal control and all results are presented as the ratio of 5 α R signal relative to the actin signal. Quantitation is performed using a scanning densitometer and values are compared to controls. FIG. 3 shows the inhibition of 5- α R type I using antisense oligonucleotide Seq. ID No. 5 relative to controls that are untreated, those receiving lipofectin, or a scrambled control oligonucleotide. FIG. 4 shows the inhibition of 5- α R type II using antisense oligonucleotide Seq. ID No. 14 relative to controls that are untreated, those receiving lipofectin, or a scrambled control oligonucleotide.

While a specific embodiment of the invention has been shown and described in detail to illustrate the application of the principles of the invention, it will be understood that the invention may be embodied otherwise without departing from such principles and that various modifications, alternate constructions, and equivalents will occur to those skilled in the art given the benefit of this disclosure. Thus, the invention is not limited to the specific embodiment

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:
TCGCCGTTGC CATCGCCAGG G 21

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
CCCCGTCGCC GTTGCCATCG C 21

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:
GGCGCTCCTC CGCCACCCCC G 21

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:
GACAGACCAG CTGGCCAGGG C 21

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:
GCGCCATTGG AAAGCTTCAA G 21

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:
GCGTATTTAG GTACTTIATTA G 21

-continued

(B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CTGCATCGCG CCGTGTTCCT C

21

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GGCACTGAAC CTGCATCGCG C

21

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

AGGATCCCCG CGGGCACCG C

21

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TGGGTCTTTG TGGCTTCAGA G

21

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GCCACATGTA CTTGGATTGC C

21

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes